Developmental Cell
Previews

# Lost Your Orientation? Find Your Way with PtdIns(3,4,5)P3!

Yannick Gachet[1] and Sylvie Tournier[1,*]
[1]LBCMCP-CNRS UMR5088, Institut d'Exploration Fonctionelle des Génomes (IFR109), University of Toulouse, 118 route de Narbonne, 31062 Toulouse, France
*Correspondence: tournier@cict.fr
DOI 10.1016/j.devcel.2007.11.008

**Faithful chromosome segregation requires correct positioning of the spindle during mitosis. In this issue of *Developmental Cell*, Toyoshima et al. describe a new mechanism for spindle orientation involving phosphatidylinositol-3,4,5-triphosphate [PtdIns(3,4,5)P3]. They found that in metaphase cells, dynactin was associated with the cortex through the actin cytoskeleton, and accumulated in the midsections in a PtdIns(3,4,5)P3-dependent manner. Thus, PtdIns(3,4,5)P3 regulates spindle orientation through dynein-dynactin motor complexes.**

In metazoan cells, specification of the division plane and correct chromosome segregation requires the accurate control of spindle orientation during mitosis. Additionally, cell type diversity can be generated by a strategy involving "regulated" orientation of the mitotic spindle, which dictates an asymmetric cell. Sachs and Hertwig initially observed that the spindle is usually positioned in the center of a cell, lying parallel to its longest axis. According to this theory, the geometry of the cell should be critical for spindle positioning. However, it is now clear that the control of mitotic spindle orientation in all eukaryotes is mainly based on the activity of either intracellular cortical cues (cell polarity cues) or extracellular cortical cues (cell-cell contacts) (Colombo et al., 2003; Gachet et al., 2004; O'Connell and Wang, 2000; Thery and Bornens, 2006; Yeh et al., 2000). For example, in polarized epithelial cells, a specific cortical region and adherens junctions predetermine the location and orientation of the spindle parallel to the epithelial plane by regulating the interaction of astral microtubules with cortical factors (Lu et al., 2001; Reinsch and Karsenti, 1994). An alternative study performed using nonpolarized cells (HeLa) growing on fibronectin-coated micropatterns showed that the spindle orients relative to the micropattern geometry (Thery and Bornens, 2006). Since HeLa cells round up during division, but remain attached to the adhesive micropattern by their retraction fibers, it is likely that certain actin-associated proteins that accumulate at the cell cortex at the ends of these fibers will guide spindle orientation with respect to the geometry of the cell. Additionally, Toyoshima and Nishida (2007) have recently described that mitotic spindles in HeLa cells are oriented parallel to the substratum by an integrin-mediated, cell-substrate adhesion-dependent mechanism, rather than in a cell-cell adhesion-dependent manner (Toyoshima and Nishida, 2007). In this model the authors show that actin, astral microtubules, and EB1, along with a type X myosin, play key roles in orienting the spindle (Toyoshima and Nishida, 2007).

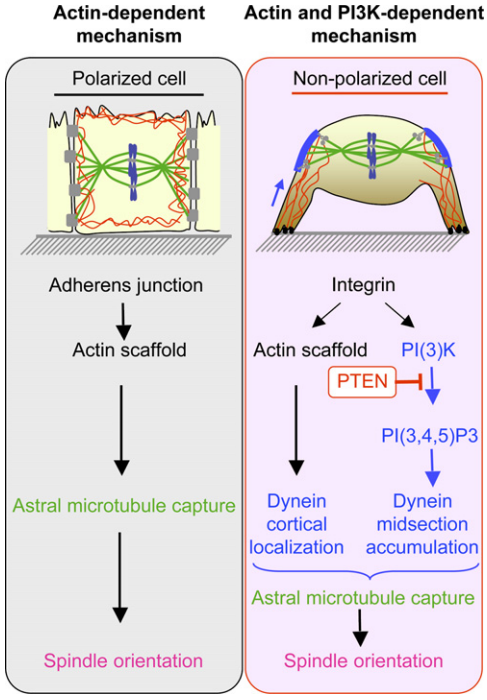


**Figure 1. Mechanisms of Spindle Orientation in Polarized or Nonpolarized Cells**
The spindle is represented in green, the actin cables in red, the chromosomes in dark blue, and the kinetochore in gray. The adherens junctions are in gray (squares in the left panel). PIP3 is represented in light blue and cortical cues such as dynein are shown in gray at the midsection of the cortex (right panel). The black dots represent integrin (right panel).

The challenge is now to identify the mechanisms underlying this integrin-dependent control of spindle orientation. In this issue of *Developmental Cell*, Toyoshima et al. (2007) show that phosphatidylinositol-3,4,5-triphosphate [PtdIns(3,4,5)P3] is essential for this process. They first show that PtdIns(3,4,5)P3 is accumulated specifically at the cortex within the midsection in metaphase cells. PI(3)K is known to be activated downstream of integrin signaling. Indeed, the authors found that β1 integrin was required for the activation

of PI(3)K in mitosis, and also for the accumulation of PtdIns(3,4,5)P3 at the cortex in metaphase cells. In order to address directly the role of PI(3)K in the control of spindle orientation, they looked at the effect of two types of PI(3)K inhibitors, LY294002 and wortmannin, on spindle orientation. Interestingly, inhibition of PI(3)K causes spindle misorientation in HeLa cells, and this defect was specifically reversed by the addition of PtdIns(3,4,5)P3, but not PtdIns(3)P, PtdIns(3,4)P2, or PtdIns(4,5)P2. Thus, exogenous PtdIns(3,4,5)P3 induces the accumulation of PI(3)K lipid products at the cortex in the midsection of mitotic cells, and restores proper spindle orientation in PI(3)K-inhibited cells. This demonstrates the requirement for PtdIns(3,4,5)P3 for correct spindle orientation parallel to the substratum.

But how does PtdIns(3,4,5)P3 regulate spindle orientation? Although obvious candidates were the astral microtubules or cortical actin structures, their localization and integrity appeared to be normal in PI(3)K-inhibited metaphase cells. The authors next investigated the role of the dynein-dynactin motor complex, a well known key player in spindle orientation in eukaryotes (Carminati and Stearns, 1997; O'Connell and Wang, 2000). They found that in metaphase cells, dynactin was associated with the cortex through the actin cytoskeleton and accumulated in the midsections of the cells in a PI(3)K-dependent and PtdIns(3,4,5)P3-dependent manner. Therefore, inhibition of PI(3)K results in dispersion of dynactin throughout the cortex, rather than its restriction to the midsection. It is likely that PI(3)K regulates spindle orientation through dynein-dynactin motor complexes, and that inhibition of PI(3)K induces dispersion of dynactin throughout the cortex, which may cause the dynein-dependent spindle rotation along the z axis, which in turn leads to spindle misorientation. Increasing the amount of PtdIns(3,4,5)P3 by depleting PTEN, a lipid phosphatase that dephosphorylates D3 of PtdIns(3,4,5)P3, also affected spindle orientation. In this situation, PtdIns(3,4,5)P3 is dispersed throughout the cortex, again leading to the dispersion of dynactin at the cortex. Therefore, when the levels of PtdIns(3,4,5)P3 are either too high or too low, the dynein-dynactin complex-dependent pulling forces generated on astral microtubules at the cortex are dispersed, resulting in the misorientation of the spindles.

In conclusion, this study illustrates the mechanisms that control spindle orientation in nonpolarized HeLa cells as opposed to polarized epithelial cells (Figure 1). Indeed, while PI(3)K and PtdIns(3,4,5)P3 are essential to control spindle orientation in HeLa cells, the authors show that they play no such role in polarized MDCK epithelial cells. They suggest that in polarized cells, spindle orientation is predetermined by the actin cytoskeleton and, as previously described, the adherens junctions (Figure 1; Reinsch and Karsenti, 1994). In agreement with this idea, adherens junctions, detected by anti-β catenin antibodies, were clearly observed by the authors in both interphase and metaphase MDCK cells, but were barely detected in HeLa cells. The actin cytoskeleton is reorganized during mitosis in HeLa cells and integrin is still present, maintaining the cell attached to the substratum with the aid of actin cables. In such nonpolarized cells, growth landmarks are not necessary to orient the spindle because the mitotic apparatus aligns parallel to the integrin underlining the actin network. Therefore, Toyoshima and colleagues suggest that the actin network emanating from integrin controls directly or indirectly the diffusion of PtdIns(3,4,5)P3, resulting in the equatorial cortical localization of the phospholipid. Since PtdIns(3,4,5)P3 is targeted for dephosphorylation by the lipid phosphatase PTEN, it will be interesting to determine how PTEN controls the spatio-temporal localization of PtdIns(3,4,5)P3. Could it be through a gradient of PTEN from the integrin to the equatorial zone? And how is the level of PtdIns(3,4,5)P3 in the midsection at the cortex adjusted?

In nonpolarized cells, the spindle position is determined by interactions between the actin network, the astral microtubules, and now, it appears, a phospholipid gradient. Toyoshima et al.'s studies establish one of the mechanisms that link the functions of the actin network and the astral microtubules during mitosis (Toyoshima and Nishida, 2007). Further exploration of the links between these networks will undoubtedly provide much-needed insight into the mechanism of spindle orientation.

## REFERENCES

Carminati, J.L., and Stearns, T. (1997). J. Cell Biol. *138*, 629–641.

Colombo, K., Grill, S.W., Kimple, R.J., Willard, F.S., Siderovski, D.P., and Gonczy, P. (2003). Science *300*, 1957–1961.

Gachet, Y., Tournier, S., Millar, J.B., and Hyams, J.S. (2004). EMBO J. *23*, 1289–1300.

Lu, B., Roegiers, F., Jan, L.Y., and Jan, Y.N. (2001). Nature *409*, 522–525.

O'Connell, C.B., and Wang, Y.L. (2000). Mol. Biol. Cell *11*, 1765–1774.

Reinsch, S., and Karsenti, E. (1994). J. Cell Biol. *126*, 1509–1526.

Thery, M., and Bornens, M. (2006). Curr. Opin. Cell Biol. *18*, 648–657.

Toyoshima, F., and Nishida, E. (2007). EMBO J. *26*, 1487–1498.

Toyoshima, F., Matsumura, S., Morimoto, H., Mitsushima, M., and Nishida, E. (2007). Dev. Cell *13*, this issue, 796–811.

Yeh, E., Yang, C., Chin, E., Maddox, P., Salmon, E.D., Lew, D.J., and Bloom, K. (2000). Mol. Biol. Cell *11*, 3949–3961.